

(19)



Europäisches Patentamt
European Patent Office
Office européen des brevets



(11)

EP 1 041 136 A1

(12)

EUROPEAN PATENT APPLICATION

(43) Date of publication:
04.10.2000 Bulletin 2000/40

(51) Int Cl.7: **C11B 5/00, A23L 1/30,
A23P 1/04**

(21) Application number: **99106106.0**

(22) Date of filing: **01.04.1999**

(84) Designated Contracting States:
**AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU
MC NL PT SE**
Designated Extension States:
AL LT LV MK RO SI

(71) Applicant: **Wacker Biochem Corporation**
Adrian, Michigan 49221 (US)

(72) Inventors:
• **Schmid, Gerhard, Dr.**
Ann Arbor, Michigan 48103 (US)

• **Harrison, Mark**
Ann Arbor, Michigan 48103 (US)
• **Polchinski, Pat**
Tecumseh, Michigan (US)

(74) Representative: **Potten, Holger et al**
Wacker-Chemie GmbH
Zentralabteilung Patente,
Marken und Lizenzen
Hanns-Seidel-Platz 4
81737 München (DE)

(54) **Process for the stabilisation of acylglycerols comprising high amounts of w-3
polyunsaturated fatty acids by means of gamma-cyclodextrin**

(57) A method to stabilize acylglycerols comprising
 ω -3 polyunsaturated fatty acids against oxidative deg-
radation characterized in that γ -cyclodextrin is mixed ei-

ther batchwise or continuously with the acylglycerol
comprising ω -3 polyunsaturated fatty acids, thus form-
ing a γ -CD/ acylglycerol complex.

EP 1 041 136 A1

Description

[0001] The invention relates to processes for the stabilization of acylglycerols comprising high amounts of ω -3 polyunsaturated fatty acids by means of γ -cyclodextrin, to the complexes thus prepared, and to their use.

[0002] Cyclodextrins are cyclic oligosaccharides which consist of 6, 7 or 8 α (1-4)-linked anhydroglucose units. The α -, β - or γ -cyclodextrins, which are prepared by, for example, enzymatic starch conversion, differ in the diameter of their hydrophobic cavity and are generally suitable for the inclusion of a large number of lipophilic substances.

[0003] Acylglycerols comprising high amounts of ω -3 polyunsaturated fatty acids as for example eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), docosatetraenoic acid (DTA), or docosapentaenoic acid (DPA) are employed in the food or dietary supplements sector to provide essential fatty acids, these essential fatty acids have been linked to the overall health and wellbeing of humans and to the treatment and prevention of diseases associated with the cardiovascular system, inflammatory disorders, human development, fitness and performance.

[0004] The main problem for the wider use of these substances is their limited stability. This results from their enhanced sensitivity to oxidative decomposition (e.g. by exposure to light, atmospheric oxygen, heat or microorganisms) due to the large number of Carbon Carbon (C-C) double bonds. Autoxidation takes place at the C-C double bond, which primarily leads to the formation of peroxides and then to aldehydes, ketones and acids. Secondary reactions involve isomerizations and polymerizations.

[0005] US 5,189,149 discloses a method of producing complexes of long chain polyunsaturated fatty acids, their salts and esters inclusive of fish and vegetable oil glycerides, with α -, β and γ -cyclodextrin (α -, β - and γ -CD) and hydroxypropyl- β -cyclodextrin, and the resulting complexes. This patent does not disclose the composition of their resulting complexes, or their stability.

[0006] The patents US 4,831,022, US 4,777,162 and US 4,775,749 disclose inclusion compounds of eicosapentaenoic acid and γ -cyclodextrin and food products containing the inclusion compound. These patents show that the complex has the highest eicosapentaenoic acid content when γ -CD is used as cyclodextrin. The patents further disclose the high stability of the eicosapentaenoic acid/ γ -cyclodextrin complex. The patents do not disclose complexes of derivatives of eicosapentaenoic acid or other ω -3 polyunsaturated fatty acids with γ -CD.

[0007] It is an object of the present invention to provide a method to stabilize acylglycerol comprising ω -3 polyunsaturated fatty acids against oxidative degradation.

[0008] This is achieved by a method in which γ -cyclodextrin is mixed with acylglycerol comprising ω -3 polyunsaturated fatty acids, either batchwise or continuously thus forming a γ -CD/ acylglycerol complex.

[0009] The batches are mixed vigorously, i.e. kneaded or stirred vigorously, depending on the consistency.

[0010] The use of γ -cyclodextrin allows better stabilization of the acylglycerols comprising ω -3 polyunsaturated fatty acids than the use of α - or β - cyclodextrin.

[0011] Preparation from concentrated aqueous γ -CD solutions has proved advantageous. The acylglycerols comprising ω -3 polyunsaturated fatty acids are added to the aqueous γ -CD solution. The CD concentration of the aqueous solution before the addition of acylglycerol is preferably between 5 and 60 % by weight. A CD concentration of 10 - 40 % by weight is especially preferred.

[0012] The weight ratio of acylglycerols comprising ω -3 polyunsaturated fatty acids to CD is preferably between 0.1 : 1 and 5 : 1, especially preferably between 0.4 : 1 and 3 : 1.

[0013] The mixing of acylglycerol and γ -CD is preferably effected in a temperature range from above freezing point to 80°C. The process is especially preferably carried out at 10 - 60°C, particularly at approx. 15 - 50°C. The mixing time depends on the temperature and is preferably between one hour and a few days. As a rule, a mixing time of 10 to 30 hours will suffice.

[0014] Complexing is preferably effected under atmospheric pressure. Complexing is preferably effected under a protective gas atmosphere (nitrogen or argon).

[0015] According to the present invention acylglycerol comprising ω -3 polyunsaturated fatty acids preferably means mono-, di-, tri- acyl, or alkyl modified glycerol, glycerol mono-phosphate, comprising at least 1 ω -3 polyunsaturated fatty acid.

[0016] ω -3 polyunsaturated fatty acid preferably means a residue selected from the group EPA, DHA, DTA or DPA.

[0017] The especially preferred meaning of acylglycerol comprising ω -3 polyunsaturated fatty acid is mono-, di-, or tri-acylglycerol, or glycerol mono- phosphate, with at least 1 EPA or DHA residue.

[0018] The most preferred meaning of acylglycerol comprising ω -3 polyunsaturated fatty acid is mono, di, tri-, acylglycerol comprising at least 1 EPA residue, in a preferred content of 5 - 30%, or at least 1 DHA residue, in a preferred content of 5 - 30%.

[0019] The composition of the ω -3 polyunsaturated fatty acids of the acylglycerols can be determined in a known manner by gas chromatographic analysis of the corresponding methyl esters.

[0020] The acylglycerols comprising ω -3 polyunsaturated fatty acids are obtained in a manner known per se, for example by wet rendering, often followed by continuous centrifugal separation of the fatty and aqueous phases. Open

hydraulic presses, cage presses and continuous screw presses are all used in the final recovery of oil from the rendering residues, and the latter are often solvent extracted after pressing.

[0021] Surprisingly, it emerged that acylglycerols comprising ω -3 polyunsaturated fatty acids can be stabilized in an outstanding manner by complexation with γ -cyclodextrin especially at EPA and/or DHA concentrations between 15% and 40% (by weight).

[0022] A markedly higher stabilization of the unsaturated compounds was found in comparison with α - and β -cyclodextrin. When tested using a Rancimat machine the γ -CD/acylglycerol complexes showed a much higher stability than those obtained with α - and β -CD.

[0023] The Gamma cyclodextrin complexes of acylglycerols comprising ω -3 polyunsaturated fatty acids have a stability time on the Rancimat machine (Induction Time) in excess of 24 hours at 100°C.

[0024] The invention therefore also relates to a complex of γ -CD and acylglycerols comprising ω -3 polyunsaturated fatty acids.

[0025] A complex according to the invention consists of Gamma cyclodextrin comprising preferably 5 - 50 % by weight of acylglycerols comprising ω -3 polyunsaturated fatty acids.

[0026] Preferred is a content of 15 % - 40 % by weight of acylglycerols comprising ω -3 polyunsaturated fatty acids.

[0027] The acylglycerols comprising ω -3 polyunsaturated fatty acids are preferably mono, di or triacylglycerols comprising at least 1 EPA in a preferred content of 5 - 30%, or at least 1 DHA residue, in a preferred content of 5 - 30%.

[0028] The complexes, which are sparingly soluble in water, can be used directly in the form of the reaction mixture. Alternatively, they can be isolated and processed by filtration, centrifugation, drying, grinding, sieving, screening, granulating or tableting to suit the procedure which is customary in each case.

[0029] The complexes according to the present invention can be used, for example, in the food or dietary supplements sector to provide essential fatty acids. Numerous studies have linked ω -3 polyunsaturated fatty acids with the treatment and prevention of disease, especially of the Cardiovascular System, and Inflammatory Disorders, e.g. decreased risk of coronary heart disease, reduction in triglyceride levels, lower blood pressure, arthritis, asthma, Crohn's disease, psoriasis. In Human Development, especially for brain and retina growth and development, and improvements in Fitness and Performance, promoting aerobic endurance and muscle recovery.

[0030] The following examples are intended to illustrate the invention in greater detail.

[0031] In the examples the stability of the complexes made was measured by the Rancimat method. The 679 Rancimat machine is an instrument for the determination of oxidative and thermal stabilities. It is produced and supplied by Metrohm Ltd. (CH-9101 Herisau, Switzerland). In the case of oils and fats or substances containing oils and fats; the stability towards oxidative decomposition can be determined. The 679 Rancimat comprises a control unit and a wet section for 3 or 6 reaction and measuring vessels. In the wet section, the samples are exposed to a stream of atmospheric oxygen at elevated temperature. In the case of oils and fats, this gives rise to organic acids. The volatile decomposition products are trapped in a measuring vessel filled with distilled water and continuously detected with a conductivity cell. The control unit assumes control and evaluation of the measurements running in the wet section.

[0032] The oxidative resistance of the acylglycerol / CD complexes was measured at 100°C. Evaluation was done using modes 1 (induction time) and 2 (stability time with ΔK set at 30 $\mu S/cm$). In general, evaluation mode 1 (induction time) was used. Airflow of 20 L/h was used for all samples. Sample quantities of 2.0 g (solid complex) and 3.5 g (liquid fish oil) were used.

[0033] Induction Time is calculated from the curve $\kappa = f(t)$. It is the time needed to reach the break point of the curve. The induction time is a characteristic of the oxidative stability of the sample under evaluation and is in almost complete agreement with the results of the time consuming AOM method. (Determination of the Oxidative Stability of Fats and Oils: Comparison between the Active Oxygen Method (AOCS Cd 12-57) and the Rancimat Method, JAOCS 63, 792 - 795 (1986), Läubli, M.W. and Bruttel, P.A.)

[0034] Stability Time is calculated from the curve $\kappa = f(t)$. It is the time needed to reach a preset conductivity change (ΔK set at 30 $\mu S/cm$).

Example 1:

[0035] To 150 ml of deionized and degassed water at 60° C in a 1-L reaction kettle was added 100.0 g of dry Gamma-cyclodextrin. The reaction vessel was shielded to exclude light and once the solids had dissolved, 22.0 g of acylglycerol solution containing approx. 5% EPA and min. 25% DHA was added. The mixture was stirred under a nitrogen atmosphere at 60° C for 24 hours then allowed to cool to ambient temperature. The resulting paste was dried under vacuum at 50°C for 48 hours. The product was obtained as a white powder in a yield of 116.0 g (95%). Acylglycerol content: 18% by weight. Oxidation Induction time at 100°C (IT_{100}): >24 hours. Stability time at 100° C: >24 hours.

Example 2:

[0036] To 150 ml of deionized and degassed water at 20° C in a 1-L reaction kettle was added 100.0 g of dry Gamma-cyclodextrin. The reaction vessel was shielded to exclude light then 22.0 g of acylglycerol solution containing approx. 5% EPA and min. 25% DHA was added and the mixture stirred under a nitrogen atmosphere at 20° C for 24 hours. The solid was removed and dried under vacuum at 50°C for 48 hours. The product was obtained as a white powder in a yield of 118.3 g (97%). Acylglycerol content: 18% by weight. Oxidation Induction time at 100°C (IT₁₀₀) : >24 hours. Stability time at 100° C: >24 hours.

10 Example 3:

[0037] To 230 ml of deionized and degassed water at 40° C in a 1-L reaction kettle was added 100.0 g of dry Gamma-cyclodextrin. The reaction vessel was shielded to exclude light and once the solids had dissolved, 43.0 g of acylglycerol solution containing approx. 5% EPA and min. 25% DHA was added. The mixture was stirred under a nitrogen atmosphere at 40° C for 24 hours then allowed to cool to ambient temperature. The resulting paste was filtered to remove water then dried under vacuum at 40°C for 48 hours. The product was obtained as a white powder in a yield of 138.2 g (97%). Acylglycerol content: 30% by weight. Oxidation Induction time at 100°C (IT₁₀₀) : >23 hours. Stability time at 100° C: >20 hours.

20 Example 4:

[0038] To 230 ml of deionized and degassed water at 40° C in a 1-L reaction kettle was added 100.0 g of dry Gamma-cyclodextrin. The reaction vessel was shielded to exclude light and once the solids had dissolved, 67.0 g of acylglycerol solution containing approx. 5% EPA and min. 25% DHA was added. The mixture was stirred under a nitrogen atmosphere at 40° C for 24 hours then allowed to cool to ambient temperature. The resulting paste was filtered to remove water then dried under vacuum at 50°C for 48 hours. The product was obtained as a white powder in a yield of 162.2 g (97%). Acylglycerol content: 40% by weight. Oxidation Induction time at 100°C (IT₁₀₀) : >9 hours. Stability time at 100° C: >9 hours.

30 Example 5:

[0039] To 450 ml of deionized and degassed water at 40° C in a Stephan Mixer was added 200.0 g of dry Gamma-cyclodextrin. The reaction vessel was shielded to exclude light and once the solids had dissolved, 67.0 g of acylglycerol solution containing approx. 5% EPA and min. 25% DHA was added. The mixture was stirred under a nitrogen atmosphere at 40° C for 24 hours then allowed to cool to ambient temperature. The resulting paste was dried under vacuum at 50°C for 48 hours. The product was obtained as a white powder in a yield of 250.4 g (94%). Acylglycerol content: 25% by weight. Oxidation Induction time at 100°C (IT₁₀₀) : >24 hours. Stability time at 100° C: >11 hours.

40 Example 6:

[0040] To 230 ml of deionized and degassed water at 40° C in a 1-L reaction kettle was added 100.0 g of dry Gamma-cyclodextrin. The reaction vessel was shielded to exclude light and once the solids had dissolved, 25.0 g of acylglycerol solution containing approx. 5% EPA and min. 25% DHA was added. The mixture was stirred under a nitrogen atmosphere at 40° C for 24 hours then allowed to cool to ambient temperature. The resulting paste was filtered to remove water then dried under vacuum at 50°C for 3 days. The product was obtained as a white powder in a yield of 121.3 g (97%). Acylglycerol content: 20% by weight. Oxidation Induction time at 100°C (IT₁₀₀) : >24 hours. Stability time at 100° C: >24 hours.

50 Example 7:

[0041] To 230 ml of deionized and degassed water at 40° C in a 1-L reaction kettle was added 100.0 g of dry Gamma-cyclodextrin. The reaction vessel was shielded to exclude light and once the solids had dissolved, 17.7 g of acylglycerol solution containing approx. 5% EPA and min. 25% DHA was added. The mixture was stirred under a nitrogen atmosphere at 40° C for 24 hours then allowed to cool to ambient temperature. The resulting paste was filtered to remove water then dried under vacuum at 50°C for 48 hours. The product was obtained as a white powder in a yield of 109.0 g (93%). Acylglycerol content: 15% by weight. Oxidation Induction time at 100°C (IT₁₀₀) : > 24 hours. Stability time at 100° C: >24 hours.

Example 8: (Comparison example)

[0042] To 50 ml of deionized and degassed water at 40° C was added 20.0 g of dry Beta-CD. The flask was covered to exclude light then 4.4 g of acylglycerol solution containing approx. 5% EPA and min. 25% DHA was added to the slurry. The mixture was stirred under a nitrogen atmosphere at 40° C for 4 hours then allowed to cool overnight to ambient temperature. The solid was collected by filtration and dried under vacuum at 40° C to give 24.0 g (98%) of material as a light yellow powder. Acylglycerol content: 18% by weight. Oxidation Induction time at 100°C (IT₁₀₀) : 3.1 hours. Stability time at 100° C: 4.4 hours.

Example 9: (Comparison example)

[0043] To 50 ml of deionized and degassed water at 40° C was added 20.0 g of dry Alpha-CD. The flask was covered to exclude light then 4.4 g of acylglycerol solution containing approx. 5% EPA and min. 25% DHA was added to the slurry. The mixture was stirred under a nitrogen atmosphere at 40° C for 4 hours then allowed to cool overnight to ambient temperature. The solid was collected by filtration and dried under vacuum at 40° C to give 11.7 g (48%) of material as a light yellow powder. Acylglycerol content: 18% by weight. Oxidation Induction time at 100°C (IT₁₀₀) : 3.4 hours. Stability time at 100° C: 4 hours.

Example 10: (Comparison example)

[0044] To 230 ml of deionized and degassed water at 40° C in a 1-L reaction kettle was added 100.0 g of dry Gamma-cyclodextrin. The reaction vessel was shielded to exclude light and once the solids had dissolved, 100.0 g of acylglycerol solution containing approx. 5% EPA and min. 25% DHA was added. The mixture was stirred under a nitrogen atmosphere at 40° C for 24 hours then allowed to cool to ambient temperature. The resulting paste was filtered to remove water then dried under vacuum at 50°C for 48 hours. The product was obtained as a yellow-white powder in a yield of 195.07 g (97%). Acylglycerol content: 50% by weight. Oxidation Induction time at 100°C (IT₁₀₀) : 6.7 hours. Stability time at 100° C: 6.8 hours.

Example 11:

[0045] To 250 ml of deionized and degassed water at 45° C in a 1-L reaction kettle was added 100.0 g of dry Gamma-cyclodextrin. The reaction vessel was shielded to exclude light and once the solids had dissolved, 24.0 g of acylglycerol solution containing approx. 25% DHA was added. The mixture was stirred under a nitrogen atmosphere at 45° C for 24 hours then allowed to cool to ambient temperature. The resulting paste was filtered to remove water then dried under vacuum at 50°C for 48 hours. The product was obtained as a white powder in a yield of 123.07 g (99%). Acylglycerol content: 20% by weight. Oxidation Induction time at 100°C (IT₁₀₀) : >24 hours.

Example 12:

[0046] To 250 ml of deionized and degassed water at 45° C in a 1-L reaction kettle was added 100.0 g of dry Gamma-cyclodextrin. The reaction vessel was shielded to exclude light and once the solids had dissolved, 24.0 g of acylglycerol solution containing approx. 25% EPA was added. The mixture was stirred under a nitrogen atmosphere at 45° C for 24 hours then allowed to cool to ambient temperature. The resulting paste was filtered to remove water then dried under vacuum at 50°C for 48 hours. The product was obtained as a white powder in a yield of 123.57 g (99%). Acylglycerol content: 20% by weight. Oxidation Induction time at 100°C (IT₁₀₀) : >24hours.

Results:

[0047] In general, complexes of acylglycerol (25 % by weight) with gamma-CD showed no oxidation over 24 hours while control samples of 18% acylglycerol mixed with alpha - or beta CD showed rapid oxidation (less than 5 hours). Complexes of acylglycerol in excess of 40% however began to exhibit oxidation.

Chart of Results

[0048]

5	Ex. No.	Acylglycerol	CD conc. (wt %)	Rxn. Temp. (C)	Rxn. Time (hr)	Yield (%)	Induct. Time 100°C (hr)	Stability Time (hr)
	1	18	40	60	24	95	>24	> 24
10	2	18	40	20	24	97	>24	>24
	3	30	30	40	24	97	>23	>20
	4	40	30	40	24	97	9.2	9.0
	5	25	30	40	24	94	>24	>11
	6	20	30	40	24	97	>24	>24
15	7	15	30	40	24	93	>24	>24
	8	18	28	40	24	99	3.1	4.4
	9	18	28	40	24	48	3.4	4
	10	50	30	40	24	97	6.7	6.8
20	11	20	30	45	24	99	>24	n.d.
	12	20	30	45	24	99	>24	n.d.
	Acylglycerol	100	-	-	-	-	5	4
	Acylglycerol/ γ -CD Mixture	~ 20	-	-	-	-	3.3	4
25	n.d. = not determined							

Claims

1. A method to stabilize acylglycerols comprising ω -3 polyunsaturated fatty acids against oxidative degradation characterized in that γ -cyclodextrin is mixed either batchwise or continuously with the acylglycerol comprising ω -3 polyunsaturated fatty acids, thus forming a γ -CD/ acylglycerol complex.
2. A method according to claim 1 characterised in that the acylglycerols comprising ω -3 polyunsaturated fatty acids are added to an aqueous γ -CD solution.
3. A method according to claim 2 characterised in that the CD concentration of the aqueous solution before the addition of acylglycerol is between 5 and 60 % by weight.
4. A method according to at least one of claims 1 to 3 characterised in a weight ratio of acylglycerols comprising ω -3 polyunsaturated fatty acids to CD between 0.1 : 1 and 5 : 1.
5. A method according to at least one of claims 1 to 4 characterised in that the mixing of acylglycerol and γ -CD is effected in a temperature range from above freezing point to 80°C.
6. A method according to at least one of claims 1 to 5 characterised in that the complexing is effected under a protective gas atmosphere (nitrogen or argon).
7. A method according to at least one of claims 1 to 6 characterised in that acylglycerol comprising ω -3 polyunsaturated fatty acids means mono-, di-, tri- acyl, or alkyl modified glycerol or glycerol mono-phosphate, with at least 1 ω -3 polyunsaturated fatty acid.
8. A method according to at least one of claims 1 to 7 characterised in that ω -3 polyunsaturated fatty acid means a residue selected from the group EPA, DHA, DTA or DPA.
9. A complex of γ -CD and acylglycerols comprising a content of 15 % - 40 % by weight of acylglycerols comprising ω -3 polyunsaturated fatty acids.

10. Use of a complex according to claim 9 in the food or dietary supplements sector.

5

10

15

20

25

30

35

40

45

50

55



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 99 10 6106

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION
Y	DE 196 12 658 A (WACKER-CHEMIE) 2 October 1997 (1997-10-02) * page 2, line 10-20; claims *	1-10	C11B5/00 A23L1/30 A23P1/04
Y	GB 2 146 650 A (HAYASHIBARA) 24 April 1985 (1985-04-24) * claims * * page 1, line 39-46 * *Experiment 1*	1-10	
D	& US 4 831 022 A		
D	& US 4 777 162 A		
D	& US 4 775 749 A		
D,X	US 5 189 149 A (T.BRUZZESE ET AL) 23 February 1993 (1993-02-23) * claims *	1-5,7-10	
A	WO 98 18610 A (B.VAN Lengerich) 7 May 1998 (1998-05-07) * page 11, line 26,27 * * page 14, line 9-12 * * page 14, line 20-26 * * page 15, line 25 * * page 16, line 6 * * claims 1,7-9,18-21,24 *	1-10	
			TECHNICAL FIELDS SEARCHED
			C11B A23L A23P
A	DATABASE WPI Section Ch, Week 8648 Derwent Publications Ltd., London, GB; Class B05, AN 86-315434 XP002115370 & JP 61 233625 A (SANRAKU OCEAN CO LTD), 17 October 1986 (1986-10-17) * abstract *	1-10	
X	EP 0 470 452 A (STAROIL) 12 February 1992 (1992-02-12) * claims *	1-5,7-10	
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 15 September 1999	Examiner Van Moer, A
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			

EPO FORM 1503 03/92 (P4/C01)

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 99 10 6106

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

15-09-1999

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE 19612658 A	02-10-1997	CA 2246282 A	09-10-1997
		WO 9736972 A	09-10-1997
		EP 0889944 A	13-01-1999
GB 2146650 A	24-04-1985	JP 60034156 A	21-02-1985
		FR 2550445 A	15-02-1985
		US 4831022 A	16-05-1989
		US 4775749 A	04-10-1988
		US 4777162 A	11-10-1988
US 5189149 A	23-02-1993	IT 1243192 B	24-05-1994
		AT 128988 T	15-10-1995
		CA 2047884 A	10-02-1992
		DE 69113713 D	16-11-1995
		DE 69113713 T	21-03-1996
		EP 0470452 A	12-02-1992
		ES 2079526 T	16-01-1996
		JP 7002662 A	06-01-1995
		NO 305034 B	22-03-1999
		PT 98606 A, B	30-06-1992
WO 9818610 A	07-05-1998	AU 4991597 A	22-05-1998
		EP 0935523 A	18-08-1999
		NO 992036 A	28-04-1999
JP 61233625 A	17-10-1986	JP 1805159 C	26-11-1993
		JP 5014686 B	25-02-1993
EP 470452 A	12-02-1992	IT 1243192 B	24-05-1994
		AT 128988 T	15-10-1995
		CA 2047884 A	10-02-1992
		DE 69113713 D	16-11-1995
		DE 69113713 T	21-03-1996
		ES 2079526 T	16-01-1996
		JP 7002662 A	06-01-1995
		NO 305034 B	22-03-1999
		PT 98606 A, B	30-06-1992
		US 5189149 A	23-02-1993

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

